REMARKS/ARGUMENTS

Reconsideration and withdrawal of the rejections of the present application are respectfully requested in view of the amendments to the claims and remarks presented herewith, which place the application into condition for allowance, or in better condition for appeal. Applicants thank Examiner Woolwine for withdrawing the prior §103(a) rejections over the combination of Nyren, Melamede, Kotewicz, and Inouye, and the aforementioned combination in addition to Myers and Malek.

Status of the Claims and Formal Matters

Claims 1-7, 9-21, and 23-35 are currently pending in this application. Claims 9-11 and 27-35 have been withdrawn from consideration as allegedly being drawn to a non-elected invention and Claims 12, 13, 19, 21, and 22 have been cancelled. Applicants reserve the right to reclaim withdrawn or cancelled subject matter in co-pending applications. By this paper, Claim 1 has been amended, without prejudice and solely to expedite prosecution pursuant to the U.S. Patent and Trademark Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). No new matter has been introduced by these amendments. Support for the amendments can be found throughout the specification as originally filed.

Rejections under 35 U.S.C. §112, 2nd paragraph

Claim 21 was rejected under 35 U.S.C. §112, 2nd paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In view of the cancellation of Claim 21, Applicants respectfully submit that this rejection is moot and should be withdrawn.

Rejections under 35 U.S.C. §112, 1st paragraph

Claims 19 and 21 were rejected under 35 U.S.C. §112, 1st paragraph as allegedly failing to comply with the written description requirement. According to the Office Action, the claims allegedly contain subject matter that was not described in the specification in such a way as to reasonably convey to the skilled artisan that the inventor had possession of the claimed invention at the time the application was filed. The Office Action contends that the claims recite that the

primer comprises dATP or ATP, wherein the dATP or ATP is exchanged for the alpha-S-analog, and appear to recite primers comprising these analogs from the beginning, rather than as a result of primer extension. In view of the cancellation of Claims 19 and 21, this rejection is moot and should be withdrawn.

Rejections under 35 U.S.C. §103(a)

Claims 1-7, 12, 14-19, and 25 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Ronaghi (PCT International Publication No. WO00/43540; hereinafter "Ronaghi") in view of Kotewicz et al (U.S. Patent no. 5,244,797; "Kotewicz") and Inouye (U.S. Patent No. 5,434,070; "Inouye").

The Office Action contends that Ronaghi allegedly teaches a method of sequencing RNA by pyrophosphate sequencing, but does not teach or disclose that the hybridization is performed in the presence of at least one RNase-inhibiting agent or that the reverse transcriptase to be used essentially lacks RNase H activity as claimed. Ronaghi is also silent regarding "recording" or the particular types of reverse transcriptases as claimed. Ronaghi is also silent regarding a mixture of reverse transcriptases, temperature, pH, nucleotide and salt concentrations as claimed. Ronaghi also fails to teach DNA primers in the context of an RNA template. Kotewicz, however, allegedly teaches a method for synthesizing cDNA from an RNA template using the reverse transcriptase derived from Moloney murine leukemia virus (MMLV), as well as the claimed temperature, pH, nucleotide, and salt concentration. Kotewicz also allegedly discloses the use of MMLV RT to perform primer extension from an RNA template using a poly-deoxythymidine primer. The Office Action argues that it would allegedly have been *prima facie* obvious to those skilled in the art to use the RT essentially lacking RNase H activity taught by Kotewicz for the purpose of sequencing an RNA molecule by the method of Ronaghi. The Office Action further alleges that it would have been obvious to use the conditions of temperature, pH, salt and nucleotide concentrations, and to use a DNA primer. Inouye was cited for allegedly disclosing the use of an RNase inhibitor when practicing the RNA methods allegedly suggested by the combined teachings of Ronaghi and Kotewicz. Applicants respectfully traverse in view of the amendments to the claims and remarks herein.

Ronaghi relates to a method of identifying a base at a target position in a sample nucleic acid sequence by use of a primer that hybridizes to the sample nucleic acid immediately adjacent to the target position. While Ronaghi discloses that such a nucleic acid sequence can be RNA, Applicants note that Ronaghi fails to teach or disclose the use of mixtures of different reverse transcriptases selected from the claimed group, or reverse transcriptases that essentially lack RNase H activity. Ronaghi also fails to teach or disclose a DNA or RNA primer used to hybridize to the RNA template, wherein the primer comprises dATP or ATP that is replaced with alpha-S-dATP, and wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer. As stated by the Office Action, Ronaghi is silent regarding DNA primers in the context of an RNA template.

Kotewicz does not cure the deficiencies of Ronaghi. Kotewicz discloses nucleotide and amino acid sequences encoding Moloney murine leukemia virus reverse transcriptase that has substantially no RNase H activity. Kotewicz is silent, however, regarding the reverse transcriptases as claimed. Kotewicz also fails to teach or disclose mixtures of the claimed reverse transcriptases, as well as the use of an RNA or DNA primer comprising ATP or dATP that is replaced with alpha-S-dATP, and wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer.

Inouye relates to msDNA molecules, which are defined at col. 6, lines 42-52 as "a molecule which comprises a branched single-stranded RNA portion which is covalently linked to a single-stranded DNA portion by a 2'-5'-phosphodiester bond between the 2'-hydroxyl group of a branched rG residue internal to the RNA strand and the 5'-phosphate of the DNA molecule." Inouye further discloses reverse transcriptases that are capable of synthesizing cDNA from a template by a 2',5'-priming event. Applicants interpret Inouye as describing a specific type of nucleic acid species and a particular type of reverse transcriptase that synthesizes those specific types of nucleic acid species that are not claimed by the present invention. Inouye fails to cure the defects of the combination of Ronaghi and Kotewicz because Inouye does not teach disclose the claimed mixture of reverse transcriptases, nor does Inouye disclose the use of a DNA or RNA primer comprising dATP or ATP that is replaced with alpha-S-dATP, and wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer.

In view of the foregoing remarks, Applicants respectfully contend that *prima facie* obviousness has not been established over the combination of Ronaghi, Kotewicz, and Inouye, because none of these references teach or disclose all of the instant claim limitations. All words in a claim must be considered in judging the patentability of that claim against the prior art. *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPQ 1970). Reconsideration and withdrawal of the §103(a) rejection over this combination of references are respectfully requested.

Claim 13 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Ronaghi in view of Kotewicz and Inouye and further in view of Wilkinson et al (Great Britain Patent Application No. GB2351559; "Wilkinson"). The Office Action concedes that none of Ronaghi, Kotewicz, or Inouye teach the use of a mixture of RNA dependent polymerases, however Wilkinson allegedly teaches such a use to increase the sensitivity and product yield of reverse transcription. The Office Action contends that it would allegedly have been *prima facie* obvious to those skilled in the art to modify the method suggested by the combined teachings of Ronaghi, Kotewicz, and Inouye by using a mixture of reverse transcriptases as allegedly proposed by Wilkinson to achieve improvements in sensitivity and yield. Applicants respectfully traverse.

For reasons discussed herein, Ronaghi, Kotewicz, and Inouye, whether considered individually or in combination, do not teach or disclose all of the instant claim limitations as required to establish *prima facie* obviousness under §103(a), particularly mixtures of the claimed reverse transcriptases essentially lacking RNase H activity, or a DNA or RNA primer that comprises dATP or ATP that is replaced with alpha-S-dATP, wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer. Wilkinson also fails to cure these deficiencies. Wilkinson discloses a specific mixture of two reverse transcriptases: avian myeloblastosis virus reverse transcriptase and reverse transcriptase derived from Moloney murine leukemia virus. The Wilkinson mixture of reverse transcriptases are presumed to contain substantial RNase H activity. There is no explicit disclosure in Wilkinson that the reverse transcriptase mixture comprises RNase H activity, but Wilkinson does disclose at page 3, lines 10-15 that, "[t]raditionally, only one reverse transcriptase, for example either AMV or MMuLV,

has been used to catalise first strand synthesis in the RT-PCR reaction. Each is characterized by distinct RNase H activities and temperature and pH optima. Principally, AMV possesses higher levels of RNase H activity relative to MMuLV, which is responsible for the degradation of the RNA in the RNA:DNA hybrids." This disclosure suggests that, while AMV and MMuLV have different RNase H activities, retaining RNase H activity in the practice of the Wilkinson invention is preferred. There is also no indication from the Wilkinson disclosure that the AMV and MMuLV are mutated in any way from the wild-type enzyme, which those skilled in the art would recognize as having RNase H activity.

For at least all of the foregoing reasons, the combination of Ronaghi, Kotewicz, Inouye and Wilkinson do not teach or disclose all of the instant claim limitations and thus fail to establish *prima facie* obviousness under §103(a). The rejection as applied to Claim 13 should be withdrawn.

Claim 20 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Ronaghi, Kotewicz, and Inouye and further in view of Myers et al (Proc. Natl. Acad. Sci. (1980) 77(3): 1316-1320; "Myers"). None of Ronaghi, Kotewicz, or Inouye teach the use of an RNA primer, however Myers allegedly teaches that reverse transcriptases can use RNA as a primer. The Office Action contends that it would allegedly have been *prima facie* obvious to the skilled artisan to use either DNA or RNA as the primer when practicing the method for sequencing RNA as allegedly taught by Ronaghi, Kotewicz, and Inouye, since both were allegedly known in the prior art to be suitable for primer extension by reverse transcriptases. Applicants traverse.

As discussed elsewhere in this Response, the combination of Ronaghi, Kotewicz, and Inouye do not teach all of the instant claim limitations as required under §103(a), because none of these references either alone or in combination teach or disclose mixtures of the claimed reverse transcriptases that essentially lack RNase H activity, or a DNA or RNA primer that comprises dATP or ATP that is replaced with alpha-S-dATP, wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer. Myers does not cure these defects. Myers teaches that RT isolated from wild-type avian myeloblastosis virus (which presumably has substantial RNase H activity) can use an RNA primer to synthesize cDNA from a single-stranded

RNA template. Notably, Myers is silent regarding the use of reverse transcriptases that essentially lack RNase H activity. Myers is also silent regarding mixtures of the instantly claimed reverse transcriptases, particularly wherein the subject of the Myers reference relates solely to experiments using avian myeloblastosis virus RT (and no other type of RT). Myers also fails to teach or suggest an RNA primer that comprises ATP and is replaced with alpha-S-dATP, wherein the alpha-S-dATP is incorporated into the extended RNA primer. Because none of the cited references Ronaghi, Kotewicz, Inouye or Myers teach each and every limitation of the instant claims, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection as applied to Claim 20.

Claims 23 and 24 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Ronaghi in view of Kotewicz and Inouye, and further in view of Malek et al (U.S. Patent No. 5,665,545; "Malek"). None of Ronaghi, Kotewicz, or Inouye teach the use of rITP in place of rGTP during RNA amplification, however Malek allegedly teaqches a method of amplifying RNA called "terminal repeat amplification method" or "TRAM", which involves the substitution of rITP for rGTP in an RNA amplification product. Such substitution presumably alleviates pausing of reverse transcription due to secondary structure formation when using the RNA in a subsequent primer extension reaction. The Office Action contends that it would allegedly have been *prima facie* obvious to use the method of RNA amplification allegedly taught by Malek when practicing the method for sequencing RNA allegedly suggested by the combined teachings of Ronaghi, Kotewicz, and Inouye. Applicants traverse.

As discussed herein, none of Ronaghi, Kotewicz, or Inouye teach mixtures of the claimed reverse transcriptases essentially lacking RNase H activity, or a DNA or RNA primer that comprises dATP or ATP that is replaced with alpha-S-dATP, wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer. Malek relates to a method for amplifying a specific nucleic acid sequence or its complement at a relatively constant temperature and without serial addition of reagents, whereby a reaction mixture comprises an RNA polymerase, a DNA polymerase, a ribonuclease that hydrolyzes RNA or an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA, in addition to ribonucleotides and deoxyribonucleotide triphosphates. The Malek method is known in the art as "TRAM" or "terminal repeat

amplification method" and involves the synthesis of an RNA/DNA duplex species from a first RNA template, which is used to synthesize a second species comprising a DNA template. This DNA template has an inverted repeat sequence that folds into a 3'-terminal stem-loop structure for self-priming. This DNA template is used thereafter to synthesize copies of the original RNA first template. Notably, Malek discloses at, for example, col. 8, lines 62-67 that the RNA of an RNA-DNA hybrid is hydrolyzed by using a ribonuclease specific for RNA-DNA hybrids, and cites ribonuclease H as an example. Malek further discloses at col. 14, lines 5-9 that "[e]ach enzyme or enzyme preparation should be free of deleterious ribonuclease ("RNase") activities, with the exception of the preferred addition of a ribonuclease activity which is specific for hybrids of an RNA and DNA (for example ribonuclease H)" (emphasis supplied by Applicants). Malek further discloses at col. 14, line 54-57 that, "[s]ince RNase H is an intrinsic activity of AMV reverse transcriptase, the *E. coli* RNase H will be supplemented in the preferred embodiment by the RNase H of AMV reverse transcriptase." Thus, the polymerases used in the Malek method contain RNase H activity, which is contrary to the instantly claimed invention.

Malek is also deficient for failing to teach or disclose mixtures of reverse transcriptases as claimed. Malek teaches mixtures of an RNA polymerase and a DNA polymerase, but not mixtures of the claimed reverse transcriptase enzymes that essentially lack RNase H activity. Malek is also silent in regard to a DNA or RNA primer that comprises dATP or ATP that is replaced with alpha-S-dATP, wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer. In view of the aforementioned deficiencies, Applicants respectfully contend that a *prima facie* case of obviousness under §103(a) has not been established, because none of the references in the cited combination teach or disclose all of the instant claim limitations. For at least all of these reasons, reconsideration and withdrawal of the §103(a) rejection over Ronaghi, Kotewicz, Inouye, and Malek are respectfully requested.

Claim 26 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Ronaghi in view of Kotewicz and Inouye and further in view of Rothberg et al (U.S. Patent No. 6,274,320; "Rothberg"). None of Ronaghi, Kotewicz, or Inouye teach determining a quantity of the RNA molecule by measuring the intensity of the incorporation signal and comparing it to a reference, however Rothberg allegedly teaches that pyrophosphate-based detection can be

calibrated by the measurement of light released following the addition of control nucleotides to the sequencing reaction mixture immediately after addition of a sequencing primer, which allegedly allows for normalization of the reaction conditions. According to the Office Action, this disclosure in Rothberg provides those skilled in the art with a method of determining how many successive identical nucleotides were incorporated, thus constituting "a quantity of the RNA molecule," and the control nucleotides constitute "a reference." Applicants disagree and traverse.

For reasons discussed elsewhere in this Response, none of Ronaghi, Kotewicz, or Inouye, whether considered separately or in combination with each other, teach or disclose all of the instant claim limitations as required under §103(a), particularly reverse transcriptases essentially lacking RNase H activity, or a DNA or RNA primer that comprises dATP or ATP that is replaced with alpha-S-dATP, wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer. Rothberg discloses a method for sequencing a nucleic acid, which includes annealing a population of circular nucleic acid molecules to a plurality of anchor primers linked to a solid support, and amplifying those members of the population of circular nucleic acid molecules which anneal to the target nucleic acid, and subsequently sequencing the amplified molecules by detecting the presence of a sequencing by-product. Rothberg, like Ronaghi, Kotewicz, and Inouye, is silent regarding mixtures of the claimed reverse transcriptases that essentially lack RNase H activity. Rothberg is also silent with regards to a DNA or RNA primer that comprises dATP or ATP that is replaced with alpha-S-dATP, wherein the alpha-S-dATP is incorporated into the extended DNA or RNA primer. Because this combination of references fail to teach or disclose all of the instant claim limitations, Applicants respectfully contend that *prima facie* obviousness under §103(a) has not been established. The rejection over Ronaghi in view of Kotewicz, Inouye, and Rothberg should be withdrawn.

CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion regarding this Response is desired, the Examiner is respectfully urged to contact the undersigned at the number given below, and is assured of full cooperation in progressing the application to allowance.

Applicants believe no additional fees are due with the filing of this Response. However, if any additional fees are required or if any funds are due, the USPTO is authorized to charge or credit Deposit Account Number: **50-0311**, Customer Number: **35437**, Reference Number: **21465-523 NATL**.

Respectfully submitted,

Dated: September 12, 2008

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